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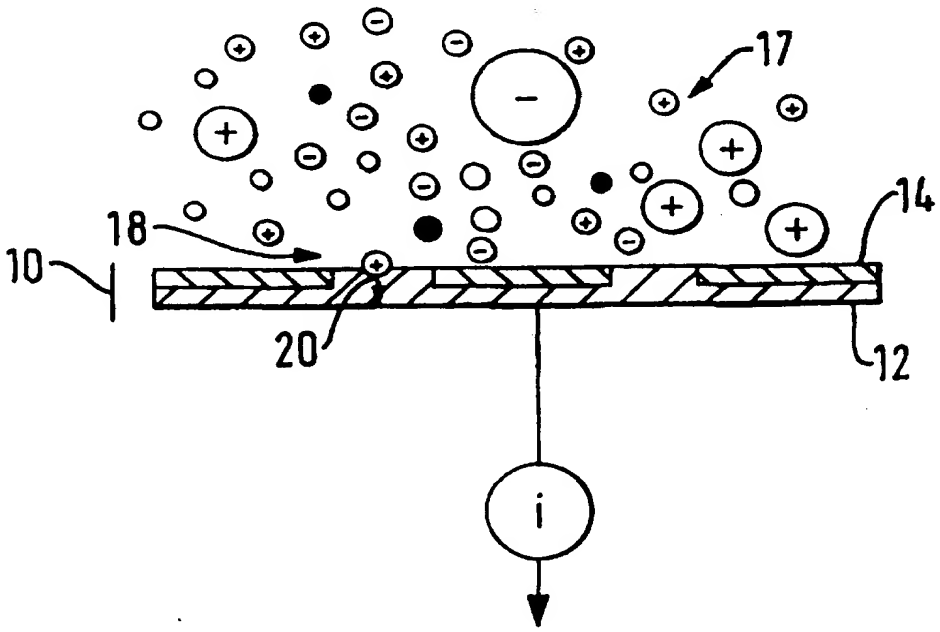
(54) Title: MICROMACHINED ION PERMEABLE COMPOSITE MEMBRANES FOR AMPEROMETRIC ION DETECTION

(57) Abstract

A micromachined ion permeable composite membrane is disclosed, which comprises a non-polarisable layer and an electrically polarisable material. The non-polarisable layer has at least one microaperture formed therethrough, and the polarisable material is disposed in relation to the microaperture(s) so as to provide a passageway of well defined cross-sectional area through the membrane for a predetermined ionic species. The membranes are particularly suited for use in amperometric ion detection and quantitation, for example in clinical diagnosis. Also disclosed are ion detectors and sensors incorporating the composite membranes of the invention.



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MICROMACHINED ION PERMEABLE COMPOSITE MEMBRANES FOR AMPEROMETRIC ION DETECTION

This invention relates to micromachined ion permeable composite membranes for the amperometric detection and quantitation of ionic species. Detection systems of the invention are applied to flow analysis and disposable ion sensing devices in clinical diagnostics and heavy metal analysis.

In the past, ion sensing membranes and transducers in the form of glass membranes and organic polymer membranes have been the major electrochemical routes for the potentiometric analysis of non-redox ionic species [1]. One common type of potentiometric probe is the glass pH electrode, which incorporates thin glass membranes, and which is used for monitoring the concentration of H^+ ions. Other membranes have been developed for a wide range of monovalent cations. The glass membranes can optionally be modified by the inclusion of hydrophobic layers to design electrodes which can specifically detect gases such as CO_2 or NH_3 [2].

These modes of ion sensing have the particular advantage of being easy to perform, but suffer from having only moderate response times of the order of several minutes or longer. The time taken to reach a steady signal is often dependent on the rate of mass transport to the surface of the sensing membrane. Further drawbacks with the potentiometric membranes are that improvements in selectivity in the presence of interfering ions can only be achieved by using elaborate ionophores such as valinomycin, which, with time, can leach out of the membrane. Furthermore, ion selective electrodes (ISE) and ion selective field effect transistors (ISFET) display a logarithmic potential-concentration response characteristic of at most 60mV and 30mV per decade of concentration for monovalent and divalent ions, respectively. Therefore electrical noise of only a few millivolts can hinder the accuracy of the measurement. The limited precision of these

type of devices also manifests itself as electrode signal drift when operated in solutions other than those defined by the manufacturer, often giving rise to variable matrix effects as a consequence.

Amperometric ion measurement techniques have several advantages over the traditional potentiometric methods. The measurement of a current signal (as a result of an ion or electron transfer process or reaction at a polarised interface between immiscible liquids) is directly proportional to the concentration of the analyte under study, and the current-time response characteristics are often of the order of seconds. Sensing probes consisting of interfaces between two immiscible liquids or between a liquid and a gel can for example be used for the amperometric monitoring of ion concentrations. Ion transfer processes and reactions through such interfaces are diffusion controlled and as such all the electroanalytical methodology developed for solid electrodes can be directly applied [3].

The interface formed with aqueous solutions and viscous hydrophobic gel materials have, for example, been used in ion sensing experiments. However, it has been found that the inherent poor electrical conductivity combined with the poor mechanical stability of these gel formulations has thus far restricted their use in experimental and sensor design [3-6]. Efforts to reduce the resistivity and to improve the mechanical stability of the gel materials have been made. For example, resistivities have been diminished by inclusion in the gel solvent of high concentrations of ionic substances such as organic electrolytes. However, the high electrical resistances associated with the aforementioned gels remain, and consequently influence the form and quantitative value of the electroanalytical signal (measured signal is visibly distorted by the effects of the large ohmic drop). The reliable identification and determination of ionic species by the principle of ion transfer at polarised polymeric interfaces (including the water to

organic gel interface) thus requires the development of a novel approach to membrane design in order to alleviate the aforementioned limitations.

We have now found that the problems associated with slab, layered and film hydrophobic gels are overcome by incorporation of a microstructured layer within a composite membrane. This design feature, which defines a regular microscopic interfacial area of well characterised geometries, produces preferentially spherical diffusion fields and concentration gradients. The microscopic nature of such mass transport fields yield a significantly diminished ohmic loss resulting from the flow of pico-ampere and nano-ampere currents. The invention therefore provides, in one aspect, a micromachined ion permeable composite membrane comprising: a non-polarisable layer having at least one microaperture formed therethrough; and an electrically polarisable material, into which a predetermined ionic species is capable of entering, the polarisable material being disposed in relation to said at least one microaperture so as to provide a passageway of well defined cross-sectional area through the sample/membrane interface for said predetermined ionic species.

Definition of the membrane structure into specific and well-defined geometric patterns may be achieved for example by UV laser photoablation or chemical etching procedures, which in effect expose parts of the polarisable material (i.e. plasticised polymer or organic gel) of the composite membrane. Such composite membranes have a number of possible applications in disposable ion sensing systems, flow injection analysis systems and immunochromatographic systems [7-10]. Also the incorporation of such composite membranes according to the invention in a probe-like assembly, in a similar manner to a potentiometric ISE, could lead to a marketable amperometric version of an ISE probe for a number of ion transferable species of interest in clinical diagnosis. An "ion transferable" species is herein defined as an ionic substance which moves from one liquid or gel

phase to another by virtue of an applied electrical potential difference across the interfacial region. The transfer phenomenon is by its nature potential specific and as such introduces a high degree of selectivity to the ion sensing system. The response characteristics of the ion permeable composite membranes of the invention as analytical transducers to determine ionic concentrations have proved to be superior to those previously described, in terms of specificity, sensitivity, limit of detection, speed of response, accuracy and precision.

In electrochemical sensors incorporating composite membranes according to the invention, the sensing component of the invention (i.e. the polarisable material), can detect and quantitate metal and molecular ions by the principle of ion transfer from the sample into the polarisable material by electric polarisation. Ion transfer currents generated by this movement of ionic species can be measured by the composite membrane transducer and are only very weakly influenced by the composite membrane resistance effects, thus significantly reducing the applied signal distortions and improving the measurement characteristics. Chemical transducers and devices consisting of the composite membranes as sensing components exhibit a high degree of selectivity for complex samples. For example, the device signal for the detection of cations remains unaffected by the presence of potentially interfering urate and ascorbate ions in clinical samples (e.g. whole blood, diluted blood fractions, serum and plasma), even when these ions are present at concentrations as high as 700μM.

The ionic response of the composite membrane (i.e. the ion transfer current) varies in direct proportion to the ion concentration of the transferable species in the sample (clinical or otherwise). The ion transfer currents flow with diffusion control and are extremely well defined because the diffusion fields are generated by the specific micromachining of the non-polarisable layer. This precision machined microstructure yields constant currents for a given

analyte concentration in electroanalytical techniques such as cyclic voltammetry and chronoamperometry. This unique diffusion field characteristic [7-10] ultimately gives the composite membrane transducer fast signal response times where the measured responses possess high signal to noise ratios. The measured signals manifest themselves as steady state voltammetric or amperometric waves, which offer several important advantages for analytical determination of ionic substances.

The invention is hereinafter described in more detail by way of example only, with reference to the accompanying drawings, in which the micromachined apertures of the illustrated embodiment are arranged in regular arrays of micro circular holes or discs. It should be stressed that other geometries are also applicable to the invention, where one of the dimensions lies on the micrometer scale, for example microsquare, microhexagon, microstrip or microband, etc.

Figure 1A is a perspective schematic view of an embodiment of micromachined ion permeable composite membrane according to the invention, wherein the reference numerals indicate: (17) water phase or sample, (18) ion transferable species, (16) micro-interfacial region, (14) non-polarisable layer, (12) polarisable layer, (19) transferred ionic species, (10) micromachined ion permeable composite membrane.

Figure 1B is a sectional schematic view, taken along line B-B of Figure 1A; wherein the reference numerals indicate: (17) water phase, (14) non-polarisable layer, (12) polarisable layer, (18) ion transferable species, (20) depiction of, for example, cationic transfer from a water phase to the polarisable layer, (10) micromachined ion permeable composite membrane.

Figure 2 is a scanning electron micrograph showing the micro aperture array of an embodiment of composite membrane

of the same general type as that illustrated in Figures 1A and 1B.

Figure 3 is a sectional schematic view of an embodiment of micromachined ion permeable composite membrane similar to that shown in Figure 1B, but having an immobilised enzyme layer, wherein the reference numerals indicate: (18) the sample, (12) polarisable layer, (22) immobilised enzyme, (24) depiction of the enzyme reaction showing the production of an ion transferable species from a non-transferable or non-interfering chemical species, (20) depiction of ionic transfer as in Figure 1.

Figure 4 is a sectional schematic view of an embodiment of micromachined ion permeable composite membrane similar to that shown in Figure 1B, but having an immobilised immunochemical reagent layer, wherein the reference numerals indicate: (18) the sample, (12) polarisable layer, (26) immobilised immunochemical reagent such as an antibody, an antibody fragment or an antigenic entity or substance, (28) depiction of an enzyme marker reaction showing the production of an ion transferable species from a non-transferable or non-interfering chemical species, (20) depiction of ionic transfer as in Figure 1.

Figure 5 is a perspective diagrammatic view of an embodiment of probe sensor incorporating a micromachined ion permeable composite membrane according to the invention, wherein the reference numerals indicate: (30) the ion permeable composite membrane, (32) replaceable tip holding the composite membrane, (34) internal probe compartment holding a reference electrode and internal electrolyte solution or gel, (36) external reference electrode, (38) body of the probe sensor.

Figure 6 is a series of cyclic voltammograms obtained from the transfer of choline across an embodiment of ion permeable composite membrane according to the invention. (This figure clearly demonstrates the advantages of the

present invention over the response of devices with large interfaces as shown in Figure 5 of reference [6]. The invention provides improvements in ohmic polarisation and measurement precision with greatly reduced background currents.).

Figure 7 is a sectional schematic view of an embodiment of micromachined ion permeable composite membrane similar to that shown in Figure 1B, and illustrates an ion sensing arrangement wherein the composite membrane of the invention lies between two electrolyte compartments (aqueous or otherwise) holding separate reference electrodes RE1 and RE2 respectively and current measuring electrodes CE1 and CE2 respectively.

Figure 8 is a schematic view of the embodiment of composite membrane shown in Figures 1 to 5, used as the detection system in a flow analysis apparatus, in which the reference numerals indicate: (54) injector, (56) detector, and (58) electrochemical workstation.

Figures 9A and 9B are, respectively, a plan view and a side view of an embodiment of disposable sensor incorporating a composite membrane of the type illustrated in Figures 1 to 5 as part of the detection system. The embodiment shown comprises an immunochromatography strip, wherein the reference numerals indicate: (40) immunochromatography strip, (42) sample window with enzyme conjugate, (44) a transfer membrane containing assays reagents, (46) detector region, (48) non-polarisable layer, (50) reference electrode such as printed silver-silver chloride ink film for the polarisable layer, (52) reference electrode such as printed silver-silver chloride ink film for the sample solution.

Figure 10 is a schematic sectional view of an embodiment of membrane according to the invention, in which the polarisable layer (60) does not fill the apertures in the non-polarisable layer (62).

Figure 11 is a schematic sectional view of an embodiment of membrane according to the invention, corresponding to Figure 10, but in which the non-polarisable layer is covered by a protective layer (64).

Figure 12 is a schematic sectional view of a further embodiment of membrane according to the invention, in which the non-polarisable layer (66) is embedded within the polarisable layer (68).

Figure 13 is a schematic sectional view of a yet further alternative embodiment of membrane according to the invention, in which an electrically conductive layer (70) is disposed between the polarisable (72) and non-polarisable (74) layers.

Referring to figures 1A and 1B, micromachined ion permeable composite membrane 10 is composed of hydrophobic ion-sensitive layer 12 and hydrophilic non-polarisable layer 14. An array of U.V. excimer machined micro-pores 16 is formed in non-polarisable layer 14 (see also Figure 2), to reveal portions of the underlying ion-sensitive layer 12. In operation the ionic analyte of interest 18 moves towards the composite membrane 10 as a result of interfacial polarisation of the membrane with respect to the sample solution, and diffusion of the ions through the exposed portions of the ion polarisable layer 16 gives rise to a measurable and well defined amperometric signal (i).

The composite membranes may be manufactured by several fabrication routes. One such approach is the lamination of a non-polarisable film (for example a polyester) with a film of the polarisable material (such as a PVC composite). This layer can be applied as an extremely thin film with micrometer thicknesses, but because of the operational requirements of the micromachined composite membrane system it is not essential to have such high fabrication control over this film thickness. The polarisable layer may be applied over the complete surface of the non-polarisable

layer, here referred to as the support material, or at selected sites thereon. Film-forming hydrophobic polymers can be used to make the polarisable layer and can be applied to the support material by several filming methods, for example extrusion coating, casting from solvents, spread filming, applying as gelled laminated film, spray coating, ink-jet printing or screen printing.

The polarisable and non-polarisable laminate may then be subjected to a laser drilling or photolithographic etching process to open up apertures (either single apertures or an array of apertures) in the non-polarisable layer. Alternatively, the micromachining of the non-polarisable layer can be carried out prior to hydrophobic polymer lamination. This procedure exposes microscopic regions of the polarisable material and defines the chemical sensing geometry of the membrane. If desired, the non-polarisable material can be pre-conditioned or surface treated by laser hydrophilisation methods in order to alter the chemical character of the surface, for example to alter the hydrophilic state of the surface. These treatments are particularly preferred steps where biochemicals such as enzymes or antibodies are to be immobilised on the surface of the non-polarisable support.

The micromachined non-polarisable layer may consist of a regular array of open regions in circular form, their size ranging from some 50nm to 500μm in diameter. Arrays of such apertures may take the form of regular hexagons or squares, etc. Single open regions may also be utilised and in this case the opening would again range from 50nm to 500μm in diameter. The open region or regions function as electrically polarisable interfaces through which ionic species transfer from the sample phase into the polarisable material. Definition of the film aperture geometries allows careful control of ion transfer flux into the composite membrane and hence the form and magnitude of the current response of the chemical sensors and detectors incorporating membranes according to the invention.

The polarisable material preferably consists of a matrix polymer which may be solely hydrophobic or have at least a substantial degree of hydrophobicity. The polarisable materials also preferably contains a solvent with plasticising properties with low water miscibility and possessing low vapour pressure. One preferred formulation for the polarisable material comprises polyvinylchloride with o-nitrophenyloctylether as plasticiser or solvent and Bis(triphenylphosphoranylidene) ammonium tetrakis(4-chlorophenylborate) electrolytic species.

In addition to the ion permeable properties, the polarisable material should have good processability, in particular the ability to adhere well to the non-polarisable layer and the ability to form stable films on that layer. For analytical purposes the material should have a sensitive and rapid response to selected ions in a sample, with good stability over the test lifetime.

The non-polarisable support is preferably composed of a polymeric film-forming material with adequate mechanical stability, low water absorption characteristics and excellent insulating properties. Such materials are usually less than 100μm in film thickness, and function as an electrically insulating surface. A wide range of commercially available film plastics with varying degrees of surface hydrophobic character may be used. Poly(ethylene terephthalate) is a suitable film material, possessing very good laser machining properties and high volume resistivity. Its excellent adherence to laminated materials allows it to be chemically functionalised and amenable to UV laser surface texturing and hydrophilisation.

Film compositions may vary depending on the processing conditions (for example whether they are formed by means of thermal, IR or UV curing procedures). Polystyrenes can be used for example to form a thin film covering over the hydrophobic layer: for example a formulation consisting of polystyrene dissolved in isophorone and applied to a surface

by the methods of extrusion coating, casting from solvents, spread filming, spray coating, ink-jet printing or screen printing and cured at low temperature forms a film with excellent dielectric and UV photoablative properties.

The formulation for the non-polarisable material may include one or more chemical reagents in order to provide a specific chemical sensor function, for example, sensors for clinical diagnostic testing. Such reagents may include enzyme substrates or enzymes. For instance, enzymes such as urease, creatinine deiminase, glutamate dehydrogenase or phenylalanine deaminase, etc. could be incorporated as part of or attached to the non-polarisable polymer component in order to provide specific clinical tests for urea, creatinine, glutamate or phenylalanine respectively.

A composite membrane with bound enzymes is shown schematically in Figure 3 for the analysis of enzyme substrates. Here, the enzymes 22 are bound to the non-polarisable layer and the enzyme-catalysed reaction producing the species to be detected is indicated by reference numeral 24. As an alternative to bound enzymes, the enzymes could be added to the test sample, for example as a dry reagent. The system could also be arranged for the analysis of enzyme concentrations where the substrate of a target enzyme is added to the test sample as a dry reagent and the enzyme quantitated by the production of selective ion transferable species.

Illustrated in figure 4 is a composite membrane functionalised with antibody reagents, which is suitable for use in immunochemical reactions and analysis. Antibodies 26 bound to the non-polarisable layer capture antigen-enzyme conjugates. The captured enzyme undergoes reaction 28, producing the species to be detected. Affinity composite membranes of this type may be used for example in enzyme immunoassays with flow instrumentation.

Formatted as chemical sensing probes, composite membranes according to the invention can be adapted to the general laboratory analysis of ionic species and biochemicals. Figure 5 is a diagrammatic representation of the features of a typical probe, in which the composite membrane 30 is contained in a replaceable tip connector 32. Internal electrolyte (gel or solution) is added to the recess between the composite membrane 34 and a suitable internal reference electrode. An external reference electrode 36 is also fitted and positioned on an exposed region on the external surface of the main body of the probe 38, so as to come into contact with a sample into which the probe is placed.

In the membranes illustrated in Figures 1, 3, 4 and 7, the polarisable layer overlies the non-polarisable layer, and also substantially fills the apertures in the non-polarisable layer. Though this geometry has been found to perform well in tests, it is by no means essential, and may readily be varied according to the requirements of the individual application for which the membrane is intended, and/or of the manufacturing process. For example, it may in a number of cases be more convenient to manufacture a membrane in which the apertured non-polarisable layer simply overlies the polarisable layer, and the polarisable layer does not extend into the apertures to any substantial extent. Such an arrangement is illustrated in Figure 10. Alternatively or additionally the non-polarisable layer may be covered by a protective layer, for example of an aqueous gel, to inhibit membrane fouling and improve sensor performance in flow systems (see Figure 11). Preferred aqueous gels include polyacrylamide, polyvinylalcohol and hydroxyethylcellulose.

A yet further alternative is illustrated in Figure 12, in which the polarisable material passes through the apertures to cover both sides of the non-polarisable layer, so that the non-polarisable layer is effectively embedded within the polarisable layer. In such an arrangement, the

thickness of the polarisable layer on the sample side of the polarisable layer should be less than the characteristic diameter of the microapertures, in order to avoid distortions to the well defined current flow provided by the presence of the microapertures. Figure 13 illustates an arrangement in which the non-polarisable layer is coated with a metallic layer (for example of gold, platinum or silver), so as to act either as a counter electrode (as indicated by CE2 in Figure 7), or as a reference electrode (as indicated by RE2 in Figure 7). In the latter case, the material of the coating is preferably anodised silver, for example AgCl, $AgSO_4$ or silver tetraphenylborate.

Other alternative geometries are feasible, the only essential requirement being the provision of an arrangement in which the ion flux through the sample/membrane interface (indicated by the arrows in Figures 10 to 13) is restricted to one or more well defined regions of the polarisable layer defined by one or more corresponding apertures in the non-polarisable layer.

The composite membranes of the invention are likely to have wide applications in clinical diagnostics. They may be designed to sense many species of clinical and environmental interest: polarisable membrane formulations are envisaged for K^+-responsive membranes, Na^+-responsive membrane, Ca^{2+}-responsive membranes, Cl^--responsive membranes, NH_4^+-responsive membranes, Pb^{2+}-responsive membranes, Cd^{2+}-responsive membranes, Zn^{2+}-responsive membranes and Cu^{2+}-responsive membranes, among others. Ion sensing agents such crown ethers, heterocyclic antibiotics, carbazone ligands (eg valinomycin for K^+) and diphenylcarbazone (for Pb^{2+} and other heavy metals) may be incorporated into the ion sensitive layer, depending on the analyte of interest. As an example of a clinical test, Figure 6 depicts a voltammogram obtained from a composite membrane used to sense and quantitate choline. Electrochemical sensors can be constructed from composite membranes according to the invention for the detection and quantification of a wide

range of blood analytes, which exhibit diminished blood matrix interferences of the type commonly encountered with conventional amperometric devices.

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CLAIMS

1. A micromachined ion permeable composite membrane comprising: a non-polarisable layer having at least one microaperature formed therethrough; and an electrically polarisable material, into which a predetermined ionic species is capable of entering, the polarisable material being disposed in relation to said at least one microaperature so as to provide a passageway of well defined cross-sectional area through the sample/membrane interface for said predetermined ionic species.

2. A micromachined ion permeable composite membrane according to claim 1, wherein the polarisable material comprises a polymer matrix.

3. A micromachined ion permeable composite membrane according to claim 2, wherein the polymer matrix is at least substantially hydrophobic in nature.

4. A micromachined ion permeable composite membrane according to claim 3, wherein the polymer matrix is of poly(vinyl chloride).

5. A micromachined ion permeable composite membrane according to any preceding claim, wherein the polarisable material comprises an organic solvent.

6. A micromachined ion permeable composite membrane according to claim 5, wherein the solvent is sparingly soluble in water, typically less than 5% in weight.

7. A micromachined ion permeable composite membrane according to claim 6, wherein the solvent is o-nitrophenyloctyl ether.

8. A micromachined ion permeable composite membrane according to any preceding claims, wherein the polarisable material comprises a hydrophobic electrolytic substance.

9. A micromachined ion permeable composite membrane according to claim 8, wherein the hydrophobic electrolytic substance is Bis(triphenylphosphoranylidene) ammonium tetrakis (4-chlorophenylborate).

10. A micromachined ion permeable composite membrane according to any preceding claim, wherein the polarisable material further comprises an ion-complexing phase transfer species.

11. A micromachined ion permeable composite membrane according to claim 10, wherein the ion complexing phase transfer species is a ligand capable of complexation with a target ion within the sample and driving the target ion into the polarisable part of the composite membrane.

12. A micromachined ion permeable composite membrane according to claim 11, wherein the non polarisable material is a polyester (e.g. poly (ethylene terephthalate)) or a vinyl polymer (e.g. polystyrene).

13. A micromachined ion permeable composite membrane according to any preceding claim, wherein the non-polarisable layer is subjected to laser hydophilisation.

14. A micromachined ion permeable composite membrane according to any preceding claim, wherein enzymes or antibodies are attached to the non-polarisable layer in the vicinity of the at least one micro aperture.

15. A micromachined ion permeable composite membrane according to any preceding claim, wherein said at least one micro aperture is formed by laser photoablation.

16. A micromachined ion permeable composite membrane according to any preceding claim, wherein the membrane possesses a single micro aperture, having a diameter of less than 500μm and more than 50nm.

17. A micromachined ion permeable composite membrane according to any of claims 1 to 15, wherein the membrane possesses an array of micro apertures.

18. A micromachined ion permeable composite membrane according to claim 17, wherein each micro aperture has a diameter of less than 500μm and more than 50nm.

19. An ion detector comprising a micromachined ion permeable composite membrane according to any preceding claim, wherein polarisation is achieved by two electrodes, one placed on each side of the membrane, the mode of detection being amperometric monitoring of the ion flux through the microaperture.

20. An ion detector according to claim 19, for use in flow injection analysis and/or chromatography.

21. A disposable ion detection apparatus comprising a micromachined ion permeable composite membrane according to any of claims 1 to 18, the membrane being directly mounted on a support arrangement.

22. An ammonia sensor comprising a micromachined ion permeable composite membrane according to any of claims 1 to 18, the membrane being covered by an ion-free hydrophilic layer and a gas permeable membrane, e.g. Teflon (poly(tetrafluoroethylene)).

23. A sensor as claimed in 22 for use in detecting urea and/or creatinine.

24. A micromachined ion permeable composite membrane according to any of claims 1 to 18, wherein the polarisable

material comprises enzymes, enzyme substrates, enzyme co-factors or the like.

25. A heavy metal ion sensor comprising: a micromachined ion permeable composite membrane according to any of claims 1 to 18; or an ion detector according to claim 19 or claim 20.

FIG. 1A

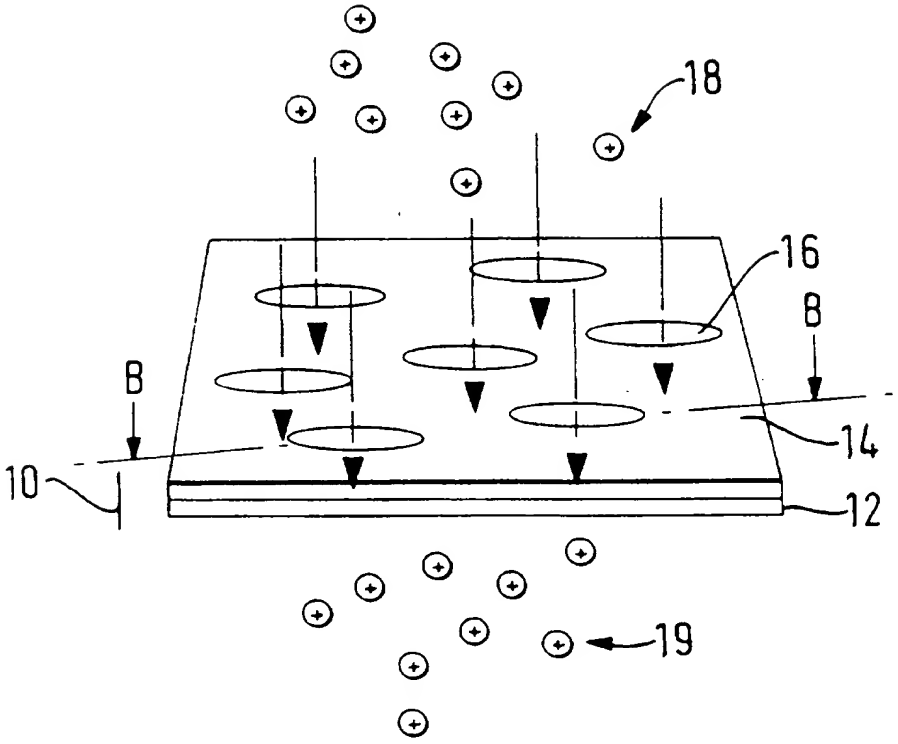
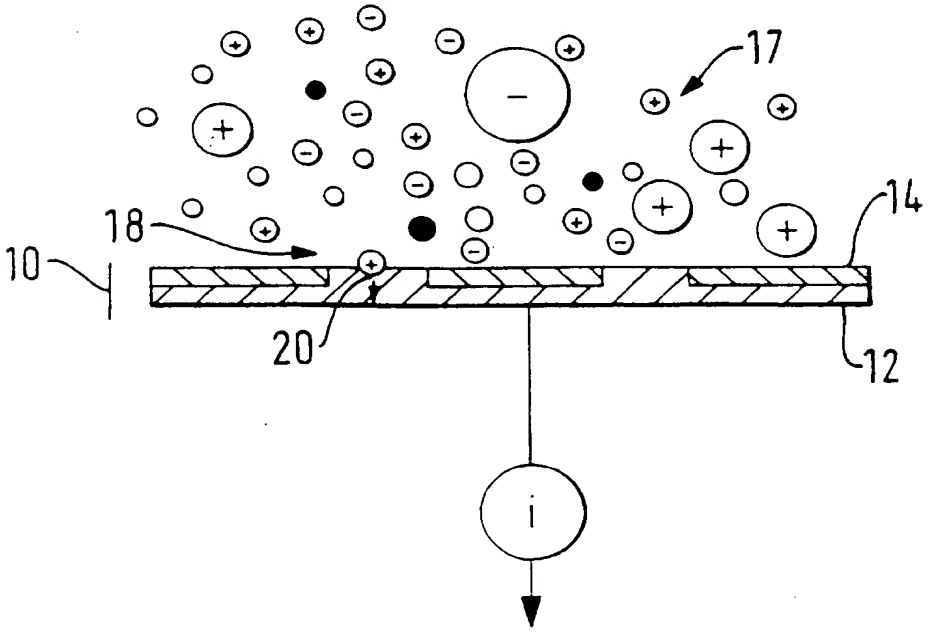


FIG. 1B



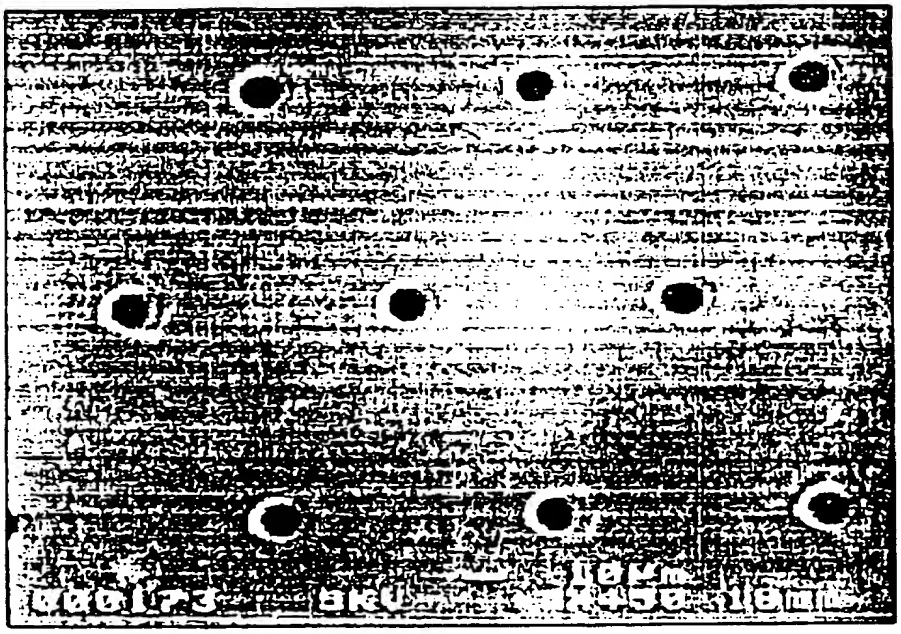


FIG. 2

FIG. 3

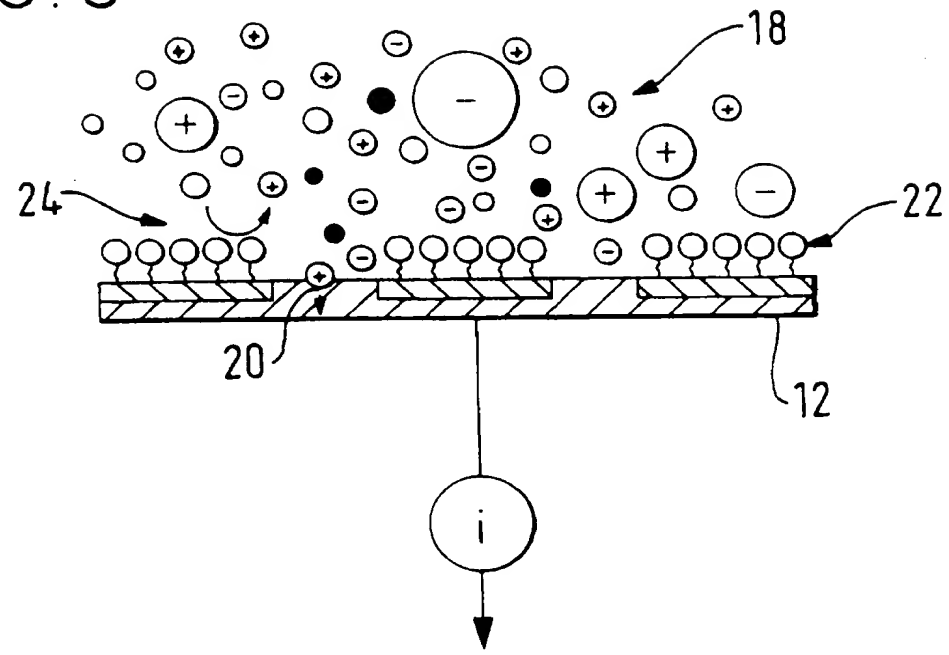
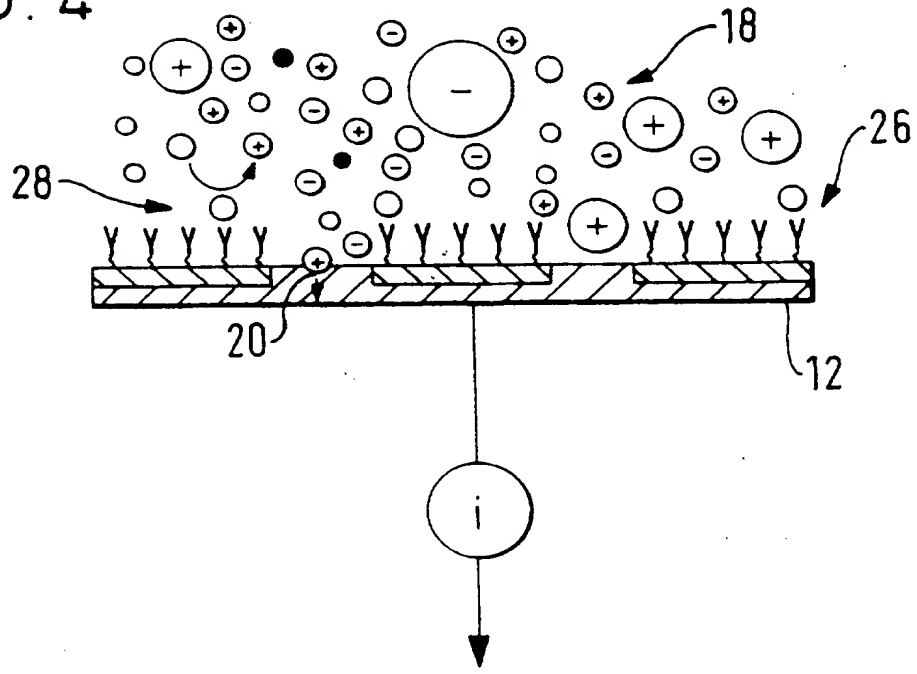


FIG. 4



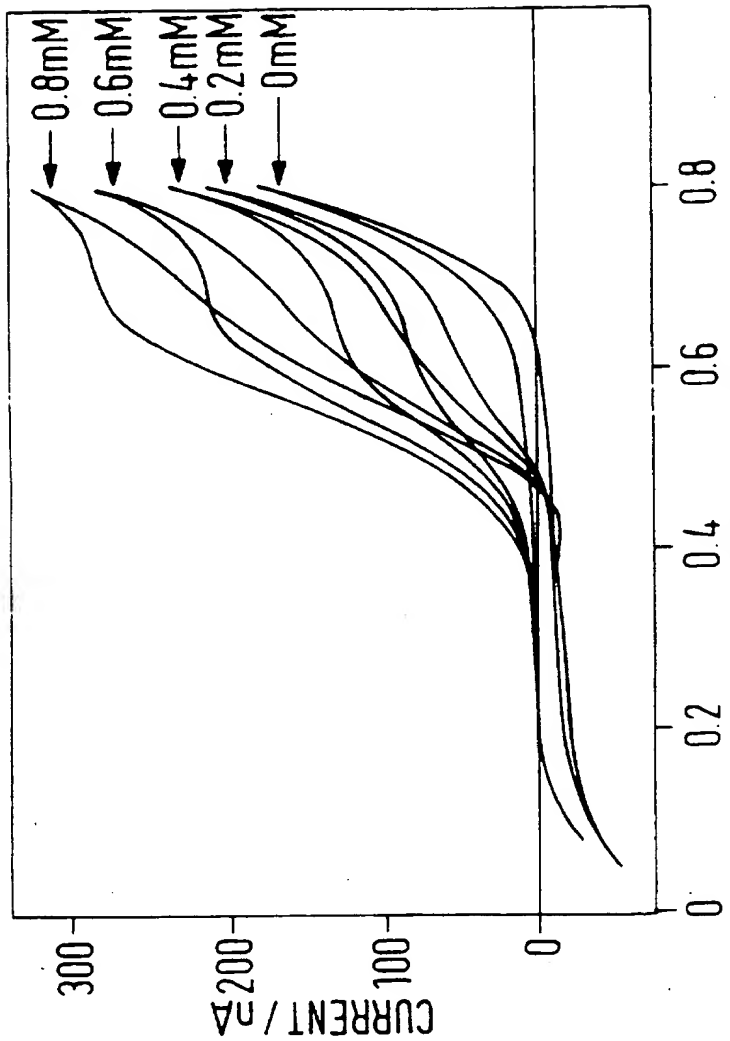

0.8mM
0.6mM
0.4mM
0.2mM
0mM
0
0.2
0.4
0.6
0.8
POTENTIAL / V
300
200
100
0
CURRENT / nA

FIG. 6

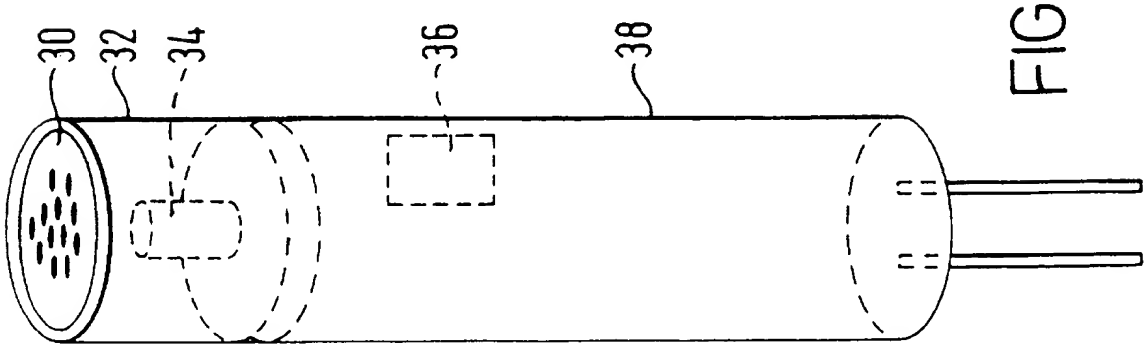

30
32
34
36
38

FIG. 5

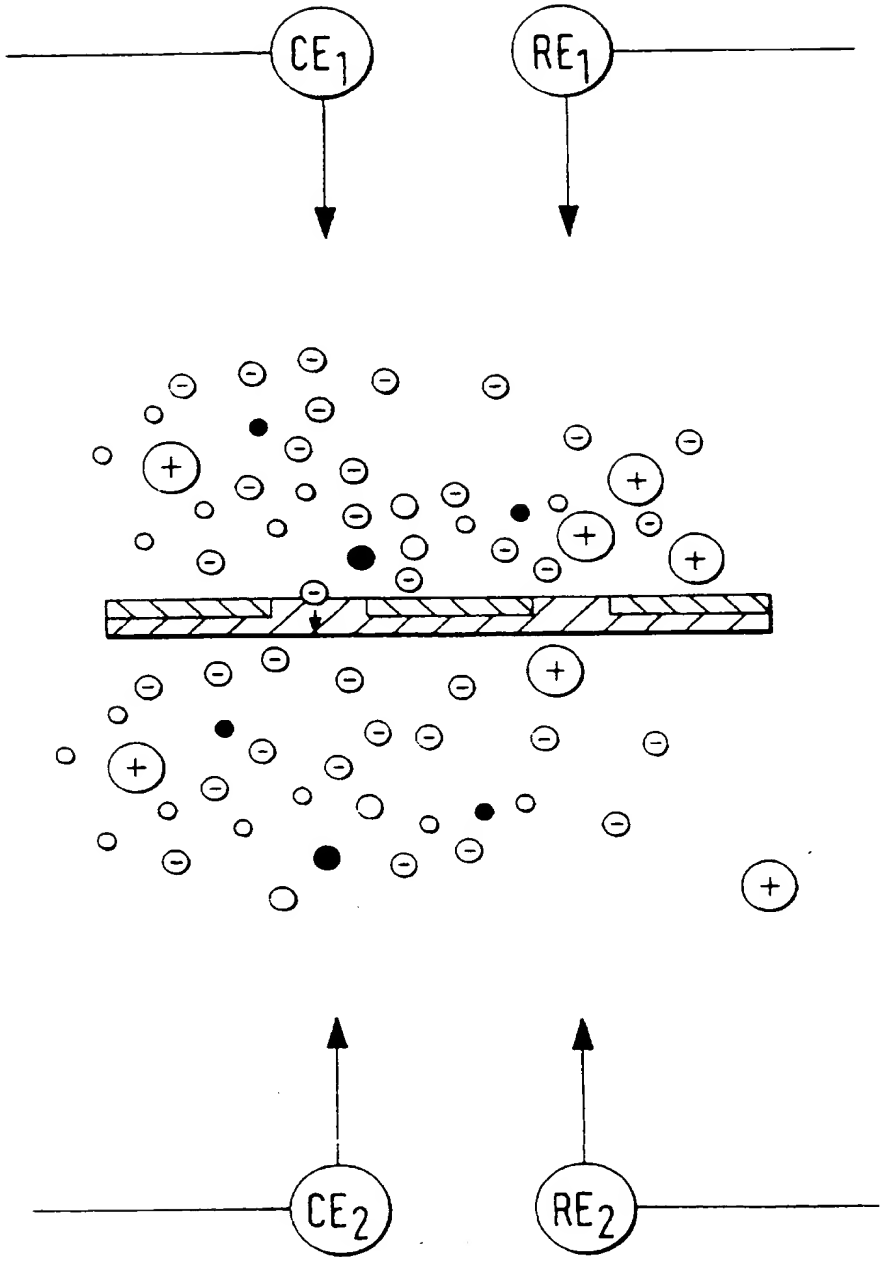


FIG. 7

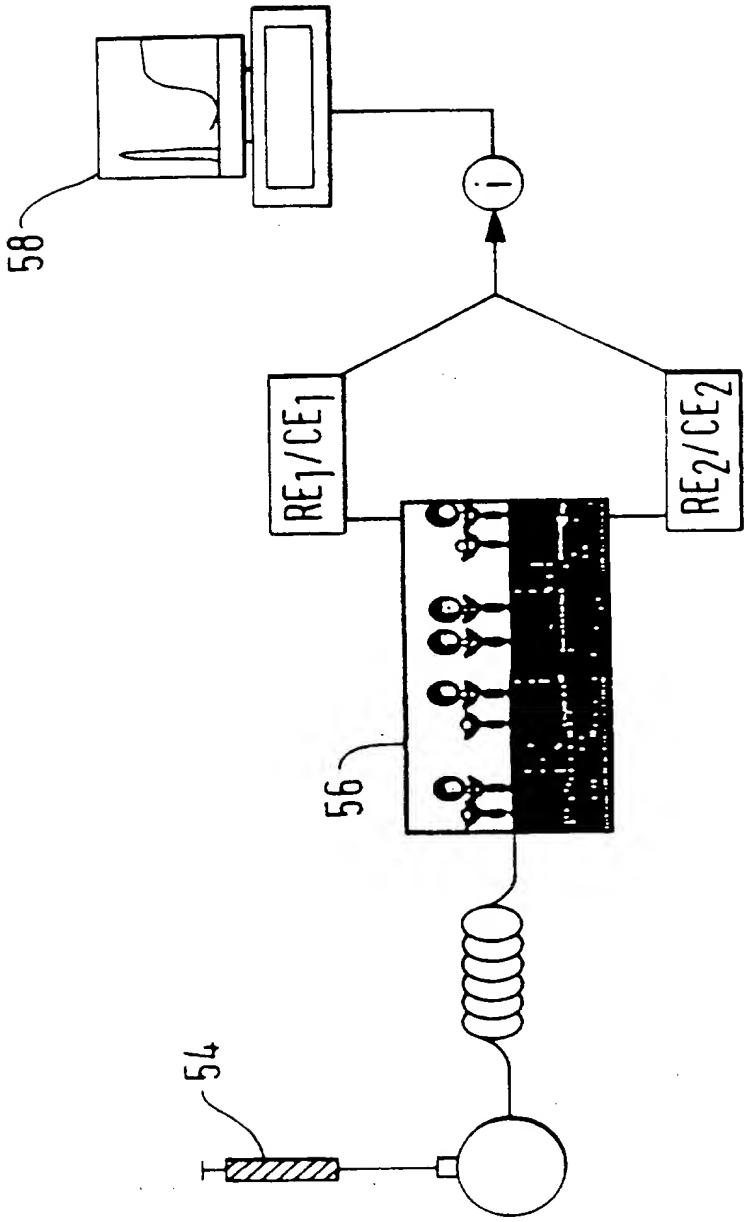


FIG. 8

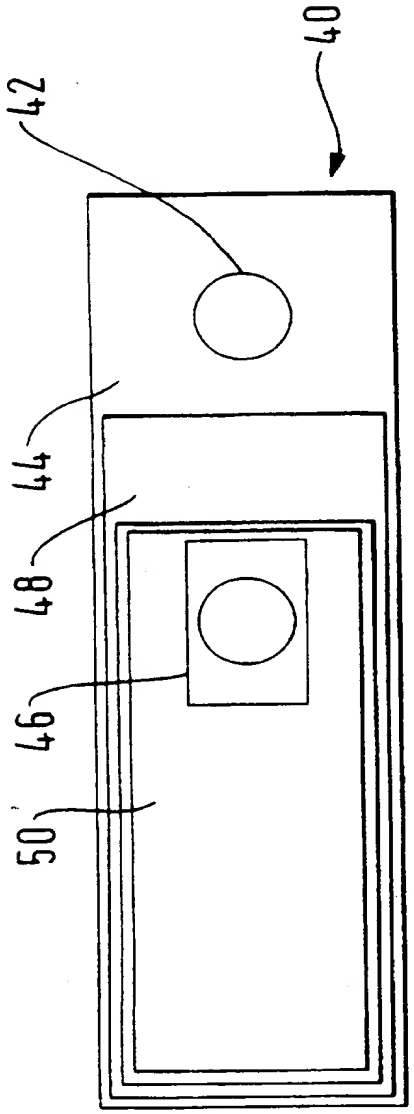


FIG.9A

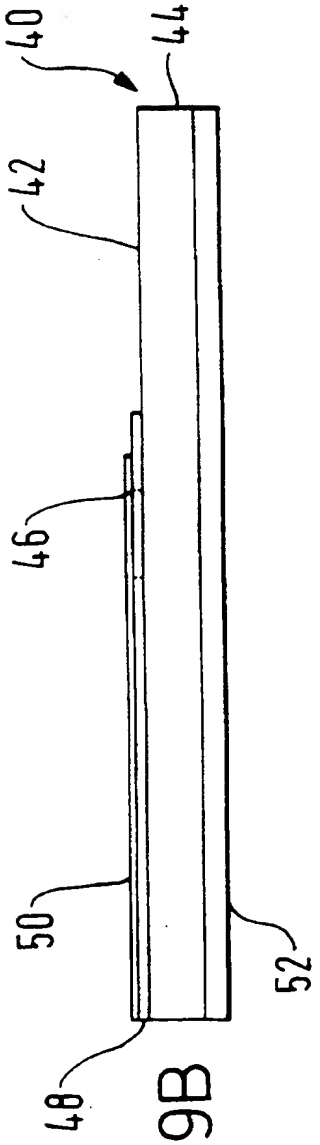


FIG.9B

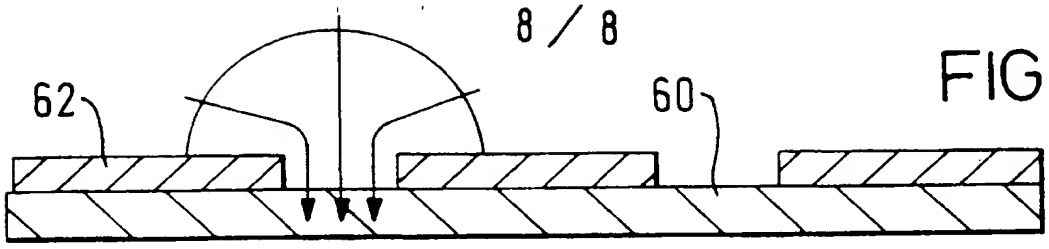


FIG.10

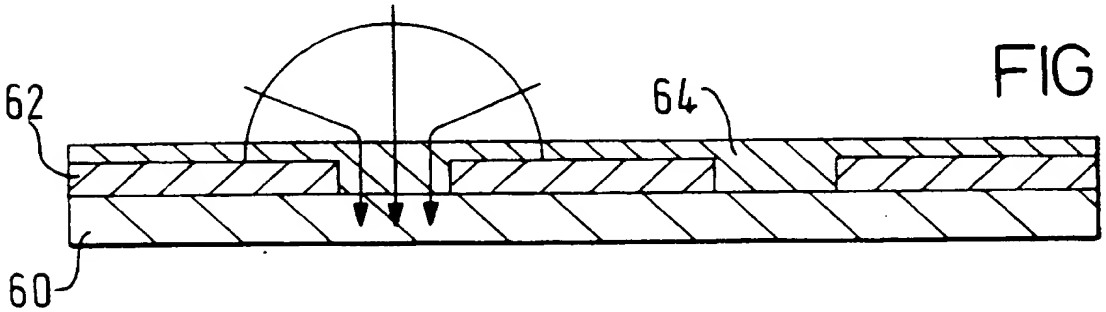


FIG.11

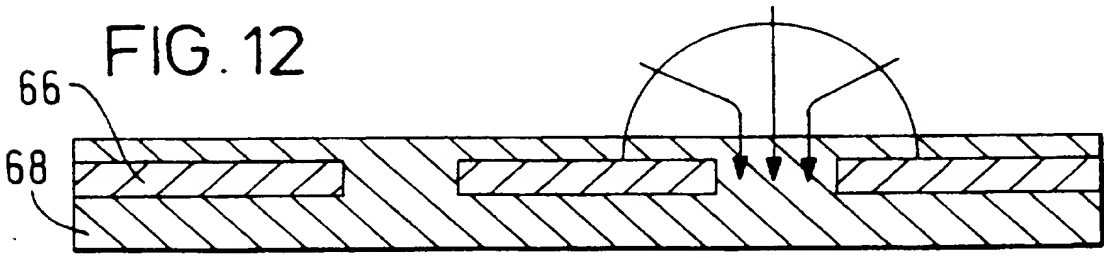


FIG.12

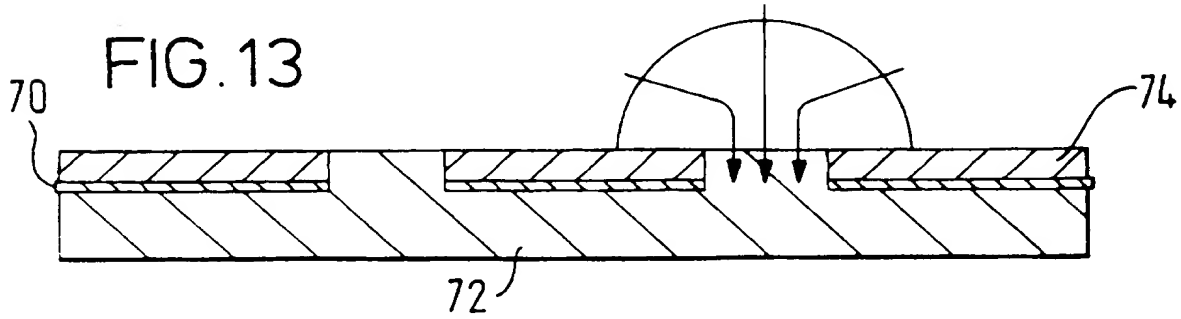


FIG.13

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/03149

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N27/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 103 031 A (HONEYWELL AND PHILIPS MEDICAL ELECTRONICS B.V.) 21 March 1984 see page 5, line 6 - line 18; figure 1 ---	1
Y	US 4 484 987 A (D. A. GOUGH) 27 November 1984 see column 5, line 51 - line 63; figure 4 ---	1
A	PATENT ABSTRACTS OF JAPAN vol. 14, no. 195 (P-1039), 20 April 1990 & JP 88 188995 A (DENKI KAGAKU KEIKI CO LTD.), 8 February 1990, see abstract ---	1
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 April 1997	17.04.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Duchatellier, M

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/03149

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ELECTROCHIMICA ACTA, vol. 39, no. 6, 1994, GB, pages 783-791, XP000646625 B. J. SEDDON: "THE APPLICATION OF EXCIMER LASER MICROMACHINING FOR THE FABRICATION OF DISC MICROELECTRODES " see abstract	1
A	ANALYTICAL SCIENCES, vol. 3, December 1987, pages 521-526, XP000646661 TOSHIYUKI OSAKAI: "A NOVEL AMPEROMETRIC AMMONIA SENSOR" cited in the application see abstract	1
A	ELECTROANALYSIS, vol. 7, no. 8, July 1995, pages 714-721, XP000646641 M. D. OSBORNE: "THE LIQUID-LIQUID MICRO-INTERFACE FOR THE AMPEROMETRIC DETECTION OF UREA" cited in the application see abstract	1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ional Application No
PCT/GB 96/03149

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 103031 A	21-03-84	CA 1202367 A	25-03-86
		JP 59081550 A	11-05-84
US 4484987 A	27-11-84	US 4650547 A	17-03-87